

Amendments to the Specification:

Please replace the paragraph bridging pages 8 and 9 with the following rewritten paragraph:

Figure 1 depicts an SDS-PAGE analysis of samples taken from the permeate streams during the extraction process for lipidated rP4, as described in Example 1 below. Lanes: 1 - Pharmacia low molecular weight markers; 2 - 0.1 μ g lipidated rP4 standard; 3 - 0.3 μ g lipidated rP4 standard; 4 - 1 μ g lipidated rP4 standard; 5 - Permeate from diafiltration with lysis buffer (10 mM Hepes, 1 mM EDTA); 6 - Permeate from diafiltration with alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) (Triton™ X-100); 7 - Permeate from diafiltration with Tris(hydroxymethyl)aminomethane (Tris™) buffer; 8 - Permeate from 1x diafiltration with n-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (Zwittergent™ 3-12) buffer; 9 - Permeate from 10x diafiltration with Zwittergent™ 3-12.

Please replace the paragraph beginning at page 9, line 8, with the following rewritten paragraph:

Figure 3 depicts an SDS-PAGE analysis of samples taken from the permeate streams during the first part of the extraction process for lipidated rP6, as described in Example 3 below. Lanes: 1 - Mark 12 standard; 2 - permeate from diafiltration with lysis buffer; 3 - Permeate from diafiltration with Triton™ X-100; 4 - Permeate from diafiltration with Tris™ buffer; 5 - Permeate from diafiltration with n-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (Zwittergent™ 3-14); 6 - Permeate from diafiltration with Zwittergent™ 3-14/0.5 M NaCl; 7 - Permeate from diafiltration with Tris™ buffer; 8 - Permeate from diafiltration with Sarcosyl.

Please replace the paragraph bridging pages 12 and 13, with the following rewritten paragraph:

The P6 protein (also known as PBOMP-1 and PAL) of *Haemophilus influenzae* has a molecular weight of approximately 15 kD and is described in United States Patent

5,110,908, which is hereby incorporated by reference. In its native form, the P6 P4 protein is lipidated. However, previous attempts to recombinantly express lipidated rP6 ~~rP4~~ resulted in low levels of expression. Copending, commonly-assigned United States Provisional Patent Application Number 60/141,067 describes an expression system which produces lipidated rP6. In order to recombinantly express the lipidated rP6 protein, the P6 gene is obtained from the bacterium and inserted into an appropriate expression vector. In examples 3 and 4 below, the expression vector pBAD18-CM was again used. The expression vector is then inserted into a suitable bacterial host cell. In examples 3 and 4 below, the host cell was again the *E. coli* BLR strain. If an inducible promoter is used, an inducer is added to cause the host cell to express the desired protein. In examples 3 and 4 below, the inducer was L-arabinose.

Please replace the paragraph beginning at page 13, line 20, with the following rewritten paragraphs:

Any protein-solubilizing detergent may be used in the extraction process including, without limitation, a ~~Zwittergent™ compound~~ zwitterionic detergent such as Zwittergent™ 3-12 or Zwittergent™ 3-14, a non-ionic ~~Triton™ compound~~ detergent such as Triton™ X-100, sarcosyl, a glucoside such as octyl-glucoside, nonyl-glucoside or decyl-glucoside, cholate or deoxycholate, or dodecyl-maltoside. In preferred embodiments, the detergents are Zwittergent™ 3-12, Triton™ X-100 and sarcosyl for the specific steps described herein.

Please insert the following new paragraph at page 13 before line 30:

The term "zwitterionic detergent" refers to a detergent that is electrically neutral overall, but has a positively charged moiety and a negatively charged moiety and is commonly used to solubilize hydrophobic proteins. The term "non-inonic detergent" refers to a molecule acting as a detergent that is uncharged.

Please replace the paragraph bridging pages 13 and 14, with the following rewritten paragraph:

A wide variety of compounds may be used as buffers in the extraction process, as long as the compound is not retained by the diafiltration membrane. Such buffers include, but are not limited to, Hepes, 3-(N-morpholino)propane sulfonic acid (MOPS), Tris™, sodium phosphate and sodium borate. In preferred embodiments, the buffers are Hepes, Tris™ and sodium phosphate for the specific steps ~~described~~ described herein.

Please replace the paragraph beginning at page 14, line 3, with the following rewritten paragraph:

Chelating agents are used at various steps of the extraction process to prevent proteolysis and/or to sequester divalent cations. The preferred chelating agent is EDTA. Divalent cations are used at various steps of the extraction process to stabilize or to solubilize the outer membrane proteins. Divalent cations include metal ions such as magnesium and calcium (Mg^{+2} and Ca^{+2}), with Mg^{+2} being preferred. Sodium chloride is the preferred salt in the salt disruption step in the process for extracting lipidated rP6.

Please replace the paragraph beginning at page 14, line 13, with the following rewritten paragraph:

The extraction may be modified by including at least one unit operation with a different diafiltration membrane having a different molecular weight cut-off, such that the lysate passes first through a larger size membrane, and then through at least one smaller size membrane. Such a sequence of membranes permits the extraction process to purify two or more integral membrane proteins separately at different stages (lysates) of the same diafiltration run.

Please replace the paragraph beginning at page 22, line 4, with the following rewritten paragraph:

(5) The lysate was diafiltered three times with 50 mM Tris™/5 mM EDTA, pH 8. This step was performed without Zwittergent™ 3-12, because ~~the Zwittergent™ compounds~~ zwitterionic detergents do not pass through the 1000 kD cut-off membrane as readily as smaller compounds such as salts. This step served to reduce the

Zwittergent™ 3-12 concentration in the membrane; the Zwittergent™ 3-12 concentration of step (4) would otherwise reduce the flow rate through the membrane during steps (6) and (8) below.

Please replace the paragraph beginning at page 22, line 18, with the following rewritten paragraph:

(7) The lysate was diafiltered two times with 50 mM Tris™/5 mM EDTA, pH 8 to again reduce the Zwittergent™ 3-12 concentration.

Please replace the paragraph beginning at page 22, line 25, with the following rewritten paragraph:

(9) The lysate was diafiltered two times with 50 mM Tris™/5 mM EDTA, pH 8 to again reduce the Zwittergent™ 3-12 concentration.

Please replace the paragraph bridging pages 22 and 23, with the following rewritten paragraph:

During the extraction, samples were taken at various points for analysis by SDS-PAGE to evaluate the effect of various diafiltration steps on the extraction of proteins. Samples were precipitated by alcohol addition, centrifuged, and then resolubilized at 20% of the original volume in SDS sample preparation buffer. This method of preparing samples concentrated the sample and reduced the Triton™ X-100 or Zwittergent™ 3-12 concentration of the samples. Triton™ X-100 or Zwittergent™ 3-12 interferes with the binding of SDS to the sample and reduces the resolution of bands on gels. Ten μ l of each sample was loaded on to Novex (Encinitas, CA) 10% acrylamide gels and the gels were run for 60-90 minutes at 125 Volts.

Please replace the paragraph beginning at page 23, line 13, with the following rewritten paragraph:

A typical SDS-PAGE analysis of the samples taken from the permeate streams during the extraction process for lipidated rP4 is shown in Figure 1. Lipidated rP4 ran at approximately 30 kD on these gels. The gel shows that some contaminating proteins were removed during diafiltration with lysis buffer (lane 5) and buffer containing Triton™ X-100 (lane 6). There was very little loss of lipidated rP4 during these diafiltration steps. During the Zwittergent™ 3-12 diafiltration step, lipidated rP4 was extracted in a partially purified state (lane 8). At the end of the Zwittergent™ 3-12 diafiltration step, very little lipidated rP4 was present in the permeate stream (lane 9). This indicated that most of the solubilized lipidated rP4 had been recovered through the permeate. Other experiments have shown that very little unsolubilized lipidated rP4 remains in the retentate after the completion of the extraction process (data not shown). The 30 kD band of the Zwittergent™ 3-12 extract has been shown to be lipidated rP4 by western analysis (data not shown).

Please replace the paragraph bridging pages 27 and 28, with the following rewritten paragraph:

(4) The lysate was diafiltered three times with 50 mM Tris™/5 mM EDTA/0.5 M NaCl/0.2% Zwittergent™ 3-14 to solubilize and remove additional proteins. NaCl was added to the buffer in this step to disrupt any ionic interactions between membrane proteins and membranes. This step was performed because lipidated rP6 is a peptidoglycan-associated lipoprotein, and the salt serves to remove membrane-bound proteins (but not lipidated rP6) from the membrane/outer membrane protein complex (lipidated rP4 is not so associated; thus this step was not performed for extracting that protein). The diafiltration was continued with three retentate volumes of 50 mM Tris™/5mM EDTA to reduce the Zwittergent™ 3-14 concentration in the retentate.